

1,079,177



PATENT SPECIFICATION

NO DRAWINGS

1,079,177

Date of Application and filing Complete Specification: Dec. 2, 1963.

No. 47469/63.

Application made in United States of America (No. 286,945) on June 11, 1963.

Complete Specification Published: Aug. 16, 1967.

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Index at acceptance:—A5 B(1S, 2S)

Int. Cl.:—A 61 k 3/00

COMPLETE SPECIFICATION

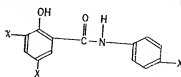
Improvements in Pesticide Compositions for Destroying Internal Worm Parasites in Animals

We: STECKER INTERNATIONAL S.P.A., an Italian corporation, of Via Turati No. 29, Milano, Italy, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to pesticide compositions for destroying internal worm parasites in animals.

The control of internal worm parasites, such as lung fluke and liver fluke, in animals, has been a perennial problem and adequate control of such parasites by prior art remedies has not been very successful.

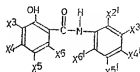
In our co-pending application No. 53924/66 (Serial No. 1,079,178) which is a divisional application from the present application there is provided a method of destroying internal worm parasites in animals comprising administering to the animal a pesticide composition, said composition comprising a veterinary carrier and a total of from 10 to 150 mg. per kg. of body weight of the animal of at least one halosalicylanilide compound of the formula:



wherein X is an atom of iodine, chlorine or bromine, and at least two of the substituents designated X are iodine atoms.

According to the present invention there is provided a method of destroying internal worm parasites in animals comprising administering to the animal a pesticide composition, said composition comprising a veterinary carrier and a total of from 5 to 150 mg. per kg. of body weight of the animal of at least one halosalicylanilide compound of the formula:

[Formula]



pound, for example glycerol. Again the carrier may be water containing a wetting agent and the composition used for water drenching the skin of the animal so that treatment of the animal with the pesticide occurs by absorption of the pesticide into the skin of the animal.

The invention is based on the discovery that certain pesticidal halosaclicyanilides, particularly the trihalosaclicyanilides, alone or in admixture with dihalosaclicyanilides are effective in the control of internal worm parasites in animals.

This discovery is illustrated in Table I which describes the results of tests carried out with brown cows infected with liver leeches (*Fasciola Hepatica* L.), treated with doses comprising a mixture of 75% 3,5,4'-tribromosaclicyanilide and 25% 5,4'-dibromosaclicyanilide, and sold commercially under the tradename of "DIAPHENE". The degree of infection of the cows by the parasite was estimated from the number of eggs in the excrement samples (Methylene-blue method by Ehrlich). If fewer than 10 eggs were found, the animals was considered "Mildly infected" (+). If the number of eggs was not higher than 15, the animal was considered "moderately infected" (++) and if the number of eggs was higher than 15, the animals was considered "badly infected" (+++). The lowest and highest number of eggs found during the test period of an animal are shown in the table in parenthesis.

After slaughter, while the body was still warm, the main bile duct and the gall bladder were removed and cut into 1 cm. thick slices. The liver leeches found were put into cups filled with 0.9% NaCl solution (38°C), and were tested for vitality and appearance. Liver leeches were considered living if movements of the mouth cone could still be detected, even though they were no longer able to move.

During the slaughter, it was detected that the bile ducts of all animals were distended, the connective tissue was thickened, and the bile ducts were moderately calcified in the area of the left liver lobe. The mucous membrane of the gall bladder showed a yellowed color.

It is a well-known fact that liver leeches cannot be found in the fresh excrement of untreated liver leeched cattle. Two normal, slightly brown-colored dead liver leeches were found 24 hours after the treatment (with 35 mg/kg. weight) in the excrement of a cow (No. 1) treated with halosaclicyanilides. *Fasciola Hepatica* eggs could not be found in the excrements of the test cows as soon as only 24 to 38 hours after beginning the treatment with halosaclicyanilides.

In untreated cattle, one very seldom finds liver leeches in the gall-bladder. This typical observation also was made with the cystic bile of the untreated test animal (No. 6). Liver leeches were always found in the gall-bladders

of cattle treated with halosaclicyanilides. Most of these were dead. The green bile of the untreated test animal (No. 6), and the one treated with halosaclicyanilides (No. 1), was clear.

In the bile ducts of untreated cows, it is well-known that only rarely are dead liver leeches ever found. A typical observation is the presence of many living and no dead liver leeches found in the bile duct of the untreated test animal (No. 6).

The bile ducts of cows treated with halosaclicyanilides showed that, in cow No. 1, more dead than live leeches were found, and in cow No. 2, no liver leeches at all were found. In cow No. 3, slaughtered very soon after treatment, it was discovered that the numerous liver leeches in the bile duct were all still alive. The dead liver leeches discovered in the gall-bladder and bile ducts of cattle treated with halosaclicyanilides were always of a light color.

The dosage given the animals did not impair their general condition, such as pulse, breathing, and body temperature. Neither a lack of appetite, nor a change in ruminating activity were encountered. During slaughter, the gall-bladders of all treated test animals, as well as of the untreated control cow, were found to be in a moderately-filled condition. Also, the livers of the treated animals failed to show any change as far as odor is concerned. The livers of all of the test animals showed that they had already been infected with *Fasciola Hepatica* for a longer period of time.

The results in Table I show that halosaclicyanilides are very effective fasciolicides in cattle. Since a dose of 20 mg/kg. of body weight is quite effective, even a smaller dose, say of 15 mg./kg., may be used. The marked effectiveness is apparent, when one notes that only 10 gms. of halosaclicyanilides are needed to destroy the worm parasites in a cow weighing 500 kg. This amount is 1/35th of the amount of hexachloroethane found to be required by Ehrlich, Lui and Winterhalter in 1957. In view of the foregoing, it does not appear necessary in many cases to employ a dosage of over 20 mg./kg. of body weight of the animal. It has been demonstrated that it is not necessary to maintain the halosaclicyanilide content of the blood at a high level for a long time in order to kill liver leeches, for dead liver leeches were discovered in the excrement 24 hours after treatment, and none, or hardly no eggs could be found.

The fact that, in the gall-bladders of halosaclicyanilide-treated cows, dead liver leeches were discovered, leads to the conclusion that the treatment has a vermifuge effect wherein the leeches are washed out of the bile ducts through the biling secretion. The fact that, in the gall-bladders of treated cows, liver leeches which moved to a large extent could be found,

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proves that surviving leeches react to the treatment by effecting an active emigration from the bile ducts.

According to the excrement test, cow No. 2 was shown to be badly infected with liver leeches before treatment. The fact that at slaughter 62 hours after treatment, no leeches were found in the bile ducts, is attributed to repeated emptying of the gall-bladder, since the treatment did not influence the food intake of the animal.

The reason for finding the dead liver leeches in the gall-bladder and live leeches in the bile duct, in cow No. 3, was that the treated cow was slaughtered only 38 hours after treatment, a time too early to effect destruction of the other parasites. Usually, a 62-hour wait before slaughtering permits an estimate of the full success of the treatment.

Discovery at slaughter of moderately-filled gall bladders confirms the clinical observation that the treatment does not impair the digestive process of the animal. Also, comparison of livers of treated and untreated animals shows that halosalicylanilide treatment does not influence the appearance or odor of the livers.

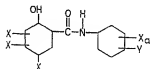
Although bromosalicylanilides were used in the tests described, it is to be understood that chloro, iodo and fluoro salicylanilides also are effective for this purpose. Although in the tests the halosalicylanilide compounds were used alone, according to the invention they are used mixed with a veterinary carrier. It may be desirable to employ with them a carrier which is a solubility improver, such as an organic hydroxy compound including glycerol, as well as with dyes, adsorbents, such as activated charcoal, or in the form of granules having coatings which function to slow down the assimilation of the granules.

Owing to the high cost of sheep and cattle, fasciolicidal tests are very expensive, and methods have been developed to reduce costs appreciably. Lienert (Wiener Tierarztlche Monatsschrift, 2, 50 (1963), p. 15, and Lienert and Jahn, *ibid.*, 2, 150 (1963), p. 213), have shown that a liver fluke implantation test involving specimens of fluke implanted beneath the skin of the back of rats, gives results correlative with those using infested cattle and serve adequately to evaluate effectiveness of agents against fascioliosis.

Such data have been obtained with 3,5-dibromo-3'-trifluoromethyl salicylanilide, using paraffinum liquidum as the vehicle. The dosage given perorally. Table II gives the results obtained in this manner. It will be observed that a dosage of 75-150 mg/kg. had an effective killing power against *Fasciola hepatica*.

Another example of a halosalicylanilide suitable for use according to the invention is 3-

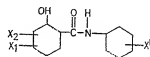
bromo-4 trifluoromethyl-4' salicylanilide. More generally, halosalicylanilides containing from one to three trifluoromethyl groups are suitable. Compounds of this type include 3', 5' bis (trifluoromethyl) salicylanilide; 2', 3', 5' tris (trifluoromethyl) salicylanilide; and 3' bromo-2', 5' bis (trifluoromethyl) salicylanilide. Suitable compounds may also fall within the general formula:



wherein X is a chlorine, bromine, iodine or hydrogen atom, Y is a trifluoromethyl group ($-\text{CF}_3$) and "a" is 1 or 2, such compounds having from one to three halogen atoms (other than those in Y) which are not adjacent to each other or to Y.

Other suitable compounds are halosalicylanilides containing one or two trifluoroalkoxy groups ($-\text{O}(\text{CH}_2)_n\text{CF}_3$) wherein n is an integer from 1 to 5. Examples of such compounds are 5-fluoro-3' trifluoromethyl-2-trifluoromethoxy salicylanilide; 3',5'-bis (trifluoromethoxy)-3- nitrosalicylanilide; and 5-fluoro-3' trifluoromethyl - 6 - trifluoroethoxy salicylanilide.

Still further suitable salicylanilide compounds are those the general formula:



wherein X is a chlorine, bromine, iodine, or fluorine atom, and X' and X'' are each a chlorine, bromine, iodine, fluorine, or hydrogen atom. Examples of these compounds are 3,5,2'-trichloro salicylanilide, 3,5-dibromo salicylanilide and 4,2'-dibromo salicylanilide.

The present treatment is applicable to domestic and fur animals, such as cattle, sheep, dogs, cats, foxes, etc. Dosing may be effected by mixing with the feed, by water drenching with the pesticide in a carrier comprising water containing a wetting agent, with capsules, bolus or soy bean pellets, or by addition to the rumen either by a long acesophagal tube or by direct injection through the flank, or by injection into the abscessum, or by subcutaneous, interperitoneal or intravascular injection.

TABLE I
Slaughter Results
Liver Leeches

Test Animal			Infection (No. of Eggs in Excrement)	Dose		Liver Lesions				Chemotherapeutic Effectiveness of Dose
No.	Age, Yrs.	Wt., Kg.		Mg./Kg. Body Wt.	Hrs. after Treatment	Bile Ducts		Gall Bladder		
						Live	Dead	Live	Dead	
1	6	393	++++ (0-22)	35	62	3	23	—	4	(+)
2	8	454	++++ (1-16)	20	62	—	—	—	2	(+)
3	10	407	+(2-7)	20	38	40	—	1	3	(+)
4	7	375	+(5-9)	Control		61	—	—	—	(-)

Note: Mildly infected = (+), moderately infected = (++) badly infected = (+++).

TABLE II

Daily Doses	Dose		Effect
	Mg./Kg.	Conc. %	
4X	150	5	+
4X	75	2	+
1X	30.7	1	—

Examples of the use of the pesticides in the compositions of the invention are as follows:—

EXAMPLE 1.

- 5 It has been found 4'-chloro-3,5-dibromosalicylanilide is effective in doses of 10, 5 and 30 mg per kilo of animal weight against *Fasciola hepatica* in sheep without any dangerous effects upon the sheep.

EXAMPLE 2.

- 10 Test conducted on the nematocidal effect of 4'-chloro-3,5-dibromosalicylanilide upon *Haemonchus contortus* in sheep have shown that this compound is effective in a dose of 30 mg per kilo of sheep weight without any dangerous effects upon the sheep.

EXAMPLE 3.

- 15 Tests on the trematocidal effect of 3,5,4'-tribromosalicylanilide on sheep have shown the compound to be effective in a dose of 20 mg per kilo of sheep weight against *Fasciola hepatica* without any dangerous effect on the sheep.

EXAMPLE 4.

- 25 Tests on the nematocidal effect of 3,5,4'-tribromosalicylanilide upon *Haemonchus contortus* in sheep have shown the compound to be effective in a dose of 50 mg per kilo of sheep weight without any dangerous effects on the sheep.

EXAMPLE 5.

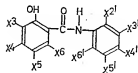
- 30 Tests on the trematocidal effect of 4-bromo-3,5-dichlorosalicylanilide has shown this compound to be effective in a dose of 20 mg per kilo of sheep weight against *Fasciola hepatica* in the sheep without any dangerous effects to the sheep.

EXAMPLE 6.

- 40 Tests on the trematocidal effect of 3,5,4'-trichlorosalicylanilide have shown the compound to be effective in a dose of 20 mg per kilo of sheep weight against *Fasciola hepatica* in sheep, without any dangerous effect on the sheep.

WHAT WE CLAIM IS:—

1. A method of destroying internal worm parasites in animals comprising administering to the animal a pesticide composition, said composition comprising a veterinary carrier and a total of from 10 to 150 mg. per kg. of body weight of the animal of at least one halosalicylanilide compound of the formula:



wherein:

X* is a chlorine, bromine, iodine or hydrogen atom,

X⁴ is a chlorine, bromine, iodine or hydrogen atom or a trifluoromethyl or nitro radical, X^{4'} is a chlorine, bromine, iodine or hydrogen atom or a trifluoromethyl radical,

X⁵ and X⁶ are each a chlorine, bromine, iodine, fluorine or hydrogen atom and may be the same or different,

X^{5'} is a chlorine, bromine, iodine or hydrogen atom or a trifluoromethyl, ethyl sulphonyl or trifluoromethoxy radical,

X^{5'} and X^{6'} are each a hydrogen atom or a trifluoromethyl radical and may be the same or different, and

X^{6'} is a hydrogen atom or a trifluoromethoxy radical, there being at least one directly-connected halogen substituent and not more than two substituents on the salicyl nucleus, and at least one substituent and not more than three substituents on the anilide nucleus.

2. A method as claimed in claim 1 wherein the total number of halogen atoms in the molecule is three.

3. A method as claimed in claim 2 wherein the halogen atoms are bromine atoms.

4. A method as claimed in claim 3 wherein the compound is 3,5,4'-tribromosalicylanilide.

5. A method as claimed in claim 1 in which the composition comprises a veterinary carrier and a total of from 5 to 150 mg. per kg. of body weight of the animal of 3,5,4'-tribromosalicylanilide in admixture with 3,4'-dibromosalicylanilide.

6. A method as claimed in claim 1 in which the compound is 3,5-dibromo-3'-trifluoromethyl salicylanilide.

7. A method as claimed in any one of the preceding claims wherein the veterinary carrier renders the composition suitable for oral administration, and the composition is administered orally.

8. A method as claimed in claim 7 in which the carrier is paraffin liquidum.

9. A method as claimed in claim 7 in which the carrier is gelatin and the composition is administered in the form of gelatin capsules containing the compound(s).

10. A method as claimed in claim 7 in which the carrier is glycerol, serving as a solubility improver.

11. A method as claimed in claim 7 in which the composition is in admixture with the animal's feed.

12. A method as claimed in any one of claims 1 to 6 in which the carrier is water containing a wetting agent and the composition is administered by drenching the skin of the animal.

13. A pesticide composition for treating internal worm parasites in animals, said composition containing as an essential active ingredient at least one compound of the formula specified in any one of claims 1 to 6 and being made up with a suitable veterinary carrier in a dosage unit form as tablets or capsules.

14. A pesticide composition as claimed in claim 13 wherein the carrier is gelatin and the composition is made up as gelatin capsules.

- 5 15. A pesticide composition for treating internal worm parasites in animals, said composition containing as an essential active ingredient at least one compound of the formula specified in any one of claims 1 to 6 and
10 being made up with paraffinum liquidum in a dosage unit form.

16. An animal feed for animals infected with internal worm parasites, said feed containing at least one compound of the formula specified in any one of claims 1 to 6 in admixture with feed stuff.

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H. D. FITZPATRICK & CO.,

Chartered Patent Agents,

3, Gray's Inn Square, London, W.C.1

and

5 Park Gardens, Glasgow, C.3.

Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press.
—1967. Published by The Patent Office, 25 Southampton Buildings, London, W.C.2,
from which copies may be obtained.